VARIATIONS IN THE RATIO IN VIVO-FLUORESCENCE/α-CHLOROPHYLL AND ITS APPLICATION TO OCEANOGRAPHY. EFFECT OF LIMITING DIFFERENT NUTRIENTS, OF NIGHT AND DAY AND DEPENDENCE ON THE SPECIES UNDER INVESTIGATION

Dolores Blasco

Translation of "Estudio de las variaciones de la relación fluorescencia *in vivo*/clorofila α, y su aplicación en oceanografía. Influencia de la limitación de diferentes nutrientes, efecto del día y noche y dependencia de la especie estudiada", Investigacion Pesquera. Vol. 37, No. 3, Dec. 1973, pp. 533 - 556.

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16. Abstract

The attempt is made to apply existing information on the fluorescence/chlorophyll a ratio to biological oceanography. Studies are performed on variations in this ratio as a function of the limitation of different nutrients, the effect of light or darkness, and the status of the ocean's phytoplankton population.

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VARIATIONS IN THE RATIO IN VIVO-FLUORESCENCE/α-CHLOROPHYLL AND ITS APPLICATION TO OCEANOGRAPHY. EFFECT OF LIMITING DIFFERENT NUTRIENTS, OF NIGHT AND DAY AND DEPENDENCE ON THE SPECIES UNDER INVESTIGATION

by Dolores Blasco

INTRODUCTION

Since the publication of Lorenzen's paper "A method for the continuous measurement of in vivo Chlorophyll concentrations" in 1966, in vivo fluorescence has become a routine measurement in biological oceanography. /533*

The method of in vivo fluorescence has in part become important and widely used because it is possible with it to determine the chlorophyll concentration without resorting to filtration and extraction; it also fits the current tendency in oceanography to automate and maximize data acquisition, to be able to obtain distribution maps of the different variables almost immediately by means of an on-board computer. In vivo fluorescence measurements are converted to chlorophyll concentrations using a factor derived from the fluorimeter calibration. The calibration consists in taking discrete water samples at the fluorimeter exit and determining their chlorophyll concentration spectrophotometrically. Usually the fluorimeter calibration is performed at the beginning of the campaign and during a short period of time; it thus frequently corresponds to a phytoplankton population of relatively homogeneous specific composition and physiological condition. The relationship observed between chlorophyll concentration and fluorescence is high and significant. Correlation coefficients are frequently greater than 0.90. Once the general validity of the method had been confirmed, no further significant time was spent attempting to carefully elucidate the actual meaning of the values.

On the other hand, the in vivo fluorescence of chlorophyll a has been extensively studied in a totally unrelated line of research, in an attempt

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*Translator's Note: Numbers in margin indicate pagination of original foreign text.

to relate it to photosynthesis mechanisms. According to those results, the relationship is not a simple one. Various forms of chlorophyll a contribute to in vivo fluorescence. Each has its characteristic emission spectrum and a different excitation spectrum. Fluorescence production differs for each of the components. In general all of these factors depend on excitation illumination intensity and other factors, such as temperature, lighting conditions to which the cells had been subjected, age of the cell, presence of photosynthesis inhibitors, etc.

The fact that additionally present pigments may also fluoresce further complicates the picture. Some transfer their excitation energy to chlorophyll a, which can thus be more or less fluorescent depending on the quantity of energy received from such pigments; some of these can furthermore show their own characteristic fluorescence bands. The fluorescence intensity due to these additional pigments is determined by processes such as energy transfer and conversion. In contrast, chlorophyll a fluorescence competes directly for the energy used in photosynthesis and can therefore provide information on the efficiency of photochemical processes.

The attempt is made in this paper to apply existing information on the ratio fluorescence/chlorophyll a to biological oceanography. With this purpose studies were performed on the variations this ratio exhibits as a function of the limitation of different nutrients, of the effect of light or darkness and the status of the ocean's phytoplankton population. The methods used were those routinely applied in oceanographic work, since the information eventually derived is to be later applied to the interpretation of data gathered during oceanographic campaigns.

MATERIALS AND METHODS

In order to be able to appreciate variations in the fluorescence/chlorophyll a ratio in sufficient detail, it became necessary, in the first place, /535 to limit as much as possible variations possibly due to the sample treatment method. A part of our work was devoted to this effort.

CHLOROPHYLL a CONCENTRATION MEASUREMENT

Chlorophyll a was determined spectrophotometrically on an acetone extract. We followed the method recommended by Scor UNESCO (J.D.J. Strickland and Parsons, 1968), which is generally used in oceanographic campaigns. There are, nevertheless, some discrepancies in the method's application. The method states, literally, "The filter may be preserved in the dark on silica gel at 1°C or below for two months, although it is better to extract the moist filter immediately and measure the extinctions without undue delay." This section explains why during campaigns -- when labor is usually in short supply, especially during hydrographic stations -- filters are customarily frozen until more time is available for extraction and readings. The period

TABLE I. DECREASE IN CHLOROPHYLL a CONCENTRATION IN FROZEN SAMPLES WHEN THE TIME BETWEEN EXTRACTION AND SPECTROPHOTOMETER READING IS INCREASED

	Time, in days	Acetone preserved filter	Frozen filter
Experiment I: initial chlorophyll a concentration: 130 µg/liter	1	0 %	44 %
	2	12	59
	6	31	31
	11	29	50
	27	40	56
	40	34	50
	46	69	65
Experiment II: initial chlorophyll a concentration: 31 µg/liter	1	0 %	26 %
	13	37	28
	13	37	28
	26	32	26
Experiment III: initial chlorophyll a concentration: 40 µg/liter	6 h	0 %	9 %
	2	25	46
	8	-	40

100% was the highest chlorophyll a value measured in each experiment

of preservation varied from occasion to occasion.

An attempt is made in this investigation to see the variations obtained when the sample was extracted immediately and when it was kept frozen. Several /536 experiments were carried out, treating the same sample in one manner or the other. Each experiment was performed several times; Table I shows the percentage decrease in chlorophyll a concentration as the filter is frozen and the time between extraction and reading increases. Samples were preserved in the dark, at 1°C.

It can be concluded that for filters preserved in acetone, the decrease as a /537 function of time is gradual, while in the case of frozen filters the decrease is due to freezing, with the concentration remaining relatively constant as a function of time. The percentage of decrease for frozen filters is not the same for all experiments; apparently the decrease depends on the rate of freezing. It is assumed that when the filters are frozen very rapidly -- for instance, using liquid nitrogen -- this decrease may not occur.

Conforming to these results, the method used in this investigation was to immerse the filter in acetone immediately following filtration; readings on the spectrophotometer were always taken within 24 hours.

The absorption spectrum was obtained with a Beckman DK-2 spectrophotometer in the earlier stages, and later with a Beckman ACTA-II spectrophotometer.

IN VIVO FLUORESCENCE MEASUREMENTS

A Turner model 111-003 fluorimeter was used for fluorescence measurements; it was equipped with a red-sensitive photomultiplier (R-136). The sample is excited with a blue fluorescence lamp, Turner 110-853. The exciting light traverses a blue filter (Turner, 110-992) with a transmission maximum at 420 mμ. The emitted light passes through a red filter (Turner 110-921) which is opaque to wavelengths below 640 mμ and has a transmission maximum at 675 mμ. Fluorescence was measured after placing the culture sample in a 1 cm diameter crystal cell. The cell is placed in the flurimeter sample holder. Since significant variations were observed in the readings of a given sample, a series of tests was performed to attempt to eliminate them and to obtain optimum conditions for measurement reproducibility. It was observed that the readings obtained from a sample carefully poured into the cell were different from those obtained when the sample was violently stirred. The stirred sample increased its value by 0.5%. By connecting a recorder to the fluorimeter, fluorescence variations as a function of time were also observed. It was furthermore observed that the sample temperature increased considerably due to the heat irradiated by the excitation lamp. Over a period of 40 minutes, the sample temperature increased from 12°C to 35°C, with the fluorescence increasing by 12.5%, see Figure 1. Although the increase may not be significant, the impossibility of establishing a plateau on the curve -- which would make it possible to establish an /538 optimum time for sample measurement -- lead us to replace the fluorimeter's standard sample holder with one operating at a regulated temperature (Turner 110-665). According to Lorenzen (1966), temperature affects the fluorescence negatively, but the author provides no information as to how the measurements were performed. Perhaps the observed increase in the fluorescence is due to the sudden shock to the cells represented by a temperature increase from 12° to 35°C in such a short period of time.

The final method used during this investigation is that followed by Papageorgiou and Govindjee (1967). A subsample of the culture is taken and left in the dark for 10 minutes; it is then carefully poured into the fluorimeter cell. Fluorescence variations as a function of time coincide with those observed by these authors, see Figure 2. The rapid initial increase is not observed, perhaps because the fluorimeter lacked a means by which to rapidly open and close off the exciting radiation; and also in part perhaps because the recorder response is not fast enough to appreciate this effect. The increase in fluorescence as a function of time is greater than that observed by the authors mentioned, but it must be taken into consideration /539 that the algae species studied were completely different. The fluorescence values used throughout this investigation are those obtained three minutes after placing the sample in the fluorimeter.

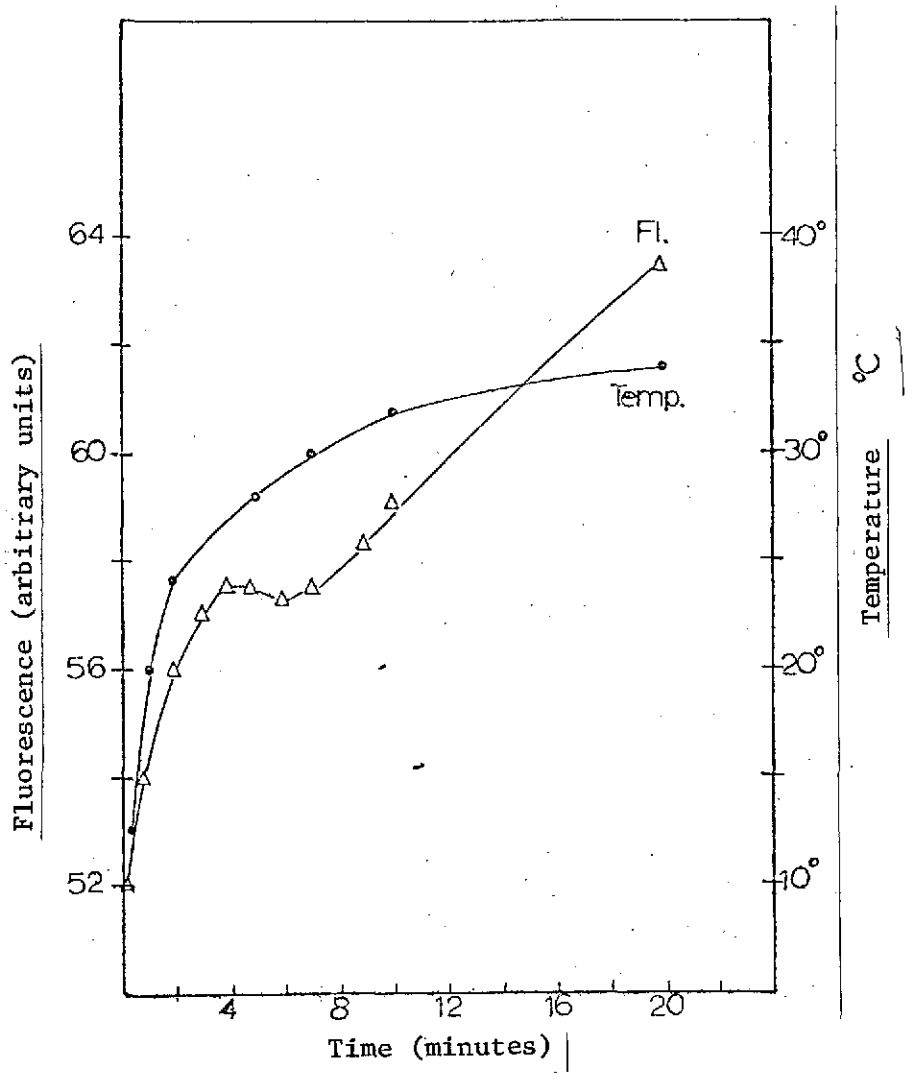

Figure 1. Variations in in vivo-fluorescence and temperature as a function of time spent by the sample in the fluorimeter.

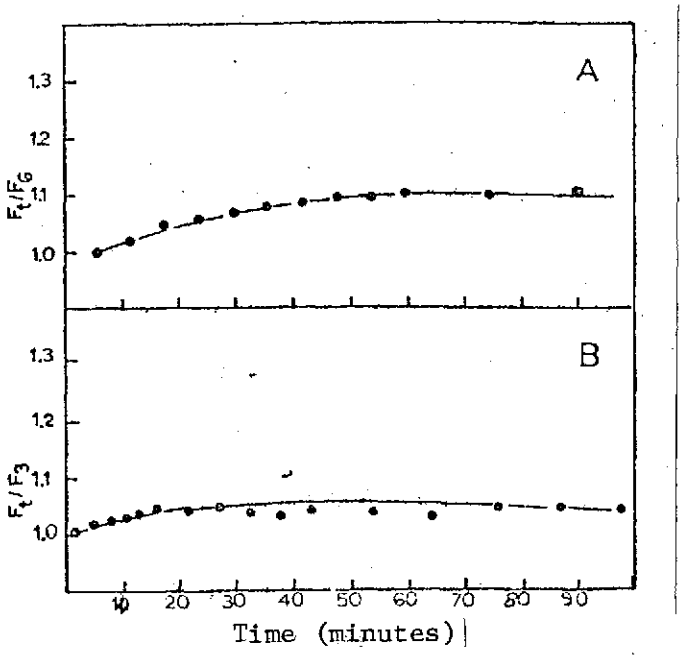

Figure 2. Changes in in vivo fluorescence as a function of time. A: Thalassiosira fluviatilis B: Anacystis nidulans (Papageorgiou and Govindjee, 1967). F_t fluorescence at minute t; F_3 and F_6 fluorescence after 3 and 6 seconds, respectively.

CULTURE METHODS

Two types of cultures were used: limited volume cultures to study the evolution of the culture as a function of time, and continuous cultures, in which the cells are always in the exponential growth phase.

The culture medium was artificial sea water, following Kester et al. (1967) (See Table II.)

The medium was sterilized by filtration through a Millipore type 0.45 HA membrane filter.

Most of the experiments were performed with the marine diatom

TABLE II. ARTIFICIAL SEA WATER (Kester et al., 1967)

$ClNa$	1.940,00 g.
SO_4Na	325,00 g.
ClK	54,90 g.
CO_3HNa	15,90 g.
BrK	7,90 g.
BO_3H_3	2,20 g.
FK	0,25 g.
$Cl_2Mg - 6H_2O$	875,20 g.
$Cl_2Ca - 2H_2O$	123,10 g.
$Cl_2Sr - 6H_2O$	2,00 g.
Distilled water	93,5 l.
Salinity	30 %

Vitamin and trace metal solution (Guillard, 1962): 2 ml

Skeletonema costatum, although some experiments were performed with the species Thalassiosira fluviatilis -- a diatom -- and Dunaliella tertiolecta, a chlorophycea.

NUTRIENT ANALYSIS

The nutrients analyzed were nitrate, phosphate, silicate and ammonia. The analyses were performed in a Technicon autoanalyzer, using the following methods: nitrate, Armstrong et al. (1967); phosphate, Murphy and Riley (1962); /540 silicate, Armstrong et al. (1967) and ammonia, Koroleff (1970).

RESULTS

Effect of Nutrients on the Ratio Fluorescence in vivo/ Unit chlorophyll

Continuous cultures

The basis of continuous cultures is the continuous addition of new medium, so that the cells are in the exponential growth phase throughout the experiment. The experiment is so conducted that all nutrients except one are supplied in excess. Population growth per unit time will then be a function of the supply of limiting component per unit time. The ratio of fluorescence to chlorophyll a was studied in various Skeletonema cultures: two with nitrogen as limiting nutrient and three with silicate as limiting component.

These two nutrients were selected because they usually limit production in the marine environment. Temperature, light and remaining nutrients were the same in all experiments. With the results thus obtained we calculated the regression lines for fluorescence in vivo and chlorophyll a. Table III shows the statistical parameters. The standard deviation of the slope refutes the hypothesis that there is no relation between the two variables, within the 99.9% confidence level.

TABLE III. STATISTICAL PARAMETERS FOR THE REGRESSION LINES FLUORESCENCE/ CHLOROPHYLL a

	Coefficient of correlation	Slope B	Intercept A	Standard deviation	N	t. calc.	t. tabul.
SiO_4-1	0,81	0.72	14,55	0.203	20	3,54	2.55
SiO_4-2	0,80	0.76	13,58	0,211	20	3.61	2.55
SiO_4-3	0,96	1,26	— 3,69	0.202	21	4.34	2.53
NH_4-1	0.73	0.53	5,55	0.145	24	3,78	2.50
NH_4-2	0,96	1,07	0,64	0,233	24	4,65	2.50

Chlorophyll a = | fluorescence x B + A |

Having observed that the slopes for these straight lines varies from experiment to experiment, the t-test was applied to verify the hypothesis /541 that the slope is different for each group (Table IV). Concentration measurements for NH_4^+ and SiO_4^- in the medium, as well as chlorophyll a concentration and chlorophyll a concentration per million cells, and culture growth rates for each experiment are shown in Table V. Table VI shows the confidence level for refuting the hypothesis that there is no difference between the measurements. According to Table IV there are no differences between the regression coefficients for experiments SiO_4^- -1 and SiO_4^- -2, while the differences are real when compared to experiment SiO_4^- -3. If we now inspect Table V, we conclude that the only common parameter between SiO_4^- -1 and SiO_4^- -2 is the chlorophyll a concentration per cell. The cause for the difference in the regression coefficient for SiO_4^- -3 can not be derived from any of the parameters studied. The only difference between SiO_4^- -1, 2 and /542 SiO_4^- -3 is the different origin of the initial population. The inoculum of the first two came from the same culture, which had recently undergone auxosporulation (a week), while experiment 3 had its origin in a population

TABLE IV. CONFIDENCE LEVEL FOR t, CALCULATED TO REFUTE THE HYPOTHESIS THAT THE TWO SLOPES ARE IDENTICAL

	SiO_4-1	SiO_4-2	SiO_4-3	SiO_4-1
NH_4-2	1,164	0.859	2.240	1,970
SiO_4-2	0,138			
	0 %			
SiO_4-3	1,542	1.388		
	90 %	90 %		
NH_4-1	1,134	0.974	0,509	
	85 %	80 %	70 %	
NH_4-2	1,164	0.899	2.240	1,970
	85 %	80 %	95 %	95 %

TABLE V. AVERAGES AND (BETWEEN BRACKETS) STANDARD DEVIATIONS FOR THE DIFFERENT PARAMETERS CHARACTERIZING EACH EXPERIMENT

	Flujo ml/min.	$NO_3^- + NH_4^-$ µg/l.	*SiO* µg/l.	*Chla.* µg/l.	*Chla/* 10^6 cels.
SiO_4-1	1.329	54,96	11.46	41,46	0.335
	(0,120)	(11,83)	(0.986)	(19,72)	(0,102)
SiO_4-2	2.324	30,24	5.97	48,60	0,352
	(0,316)	(28,51)	(4.66)	(27,99)	(0.117)
SiO_4-3	2,458	24.00	4.97	64,45	0.392
	(0,282)	(21.34)	(3.37)	(38,36)	(0,124)
NH_4-1	2,477	0,74		12,29	0,165
	(0,025)	(1.17)		(4,52)	(0,073)
NH_4-2	4,228	2.64		7.58	0,321
	(0,556)	(1,43)		(5,76)	(0,113)

that had been in vegetative reproduction for a long time (over two months). No information was found in the literature regarding the physiological characteristics of diatoms in the different stages of their reproductive cycle. According to P. Harrison (private communication), recently auxosporulated, young populations show a higher growth rate.

The difference between the slopes for the two cultures with ammonia as

TABLE VI. CONFIDENCE LEVELS TO REFUTE THE HYPOTHESIS THAT THE DIFFERENCE BETWEEN THE VARIOUS PARAMETER AVERAGES IS NULL.

	Flujo	*(NO_3^- NH_4)*	*(SiO_4^-)*	*Chl. a*	*Chl. a/10^6 cels.*
SiO_4-1					
SiO_4-2	95 %	95 %	95 %	60 %	30 %
SiO_4-2					
SiO_4-3	80 %	40 %	40 %	95 %	70 %
SiO_4-2					
SiO_4-3	95 %	95 %	95 %	95 %	80 %
NH_4-1					
NH_4-2	95 %	95 %		95 %	95 %

the limiting nutrient is significant. The origin of the inoculum for both experiments is the same, but the NH_4^- concentration, growth rate, chlorophyll concentration and chlorophyll concentration per cell are completely different. In experiment NH_4^- -1, nitrogen is really limiting and the chlorophyll a concentration per cell is smaller.

If the slopes of the cultures with limiting silicate are compared to those of the cultures with limiting ammonia, the differences are quite significant, even though on a statistical basis only the differences between NH_4^- -2 and SiO_4^- -3 are assured. It is precisely these experiments that most characteristically represent the limitation of each of these nutrients.

LIMITED VOLUME CULTURES

The purpose of this type of culture was to observe the ratio fluorescence/chlorophyll a when the cells achieve the stationary growth phase due to exhaustion of one of the nutrients in the medium. Experiments were performed with nitrate and with phosphate as the limiting nutrients. Nitrate was chosen because it is directly linked to pigment formation within the cells, and phosphate as an element of comparison. The following were analyzed in all experiments: phosphate, silicate, nitrate and ammonia concentrations in the medium; number of cells, chlorophyll a and fluorescence.

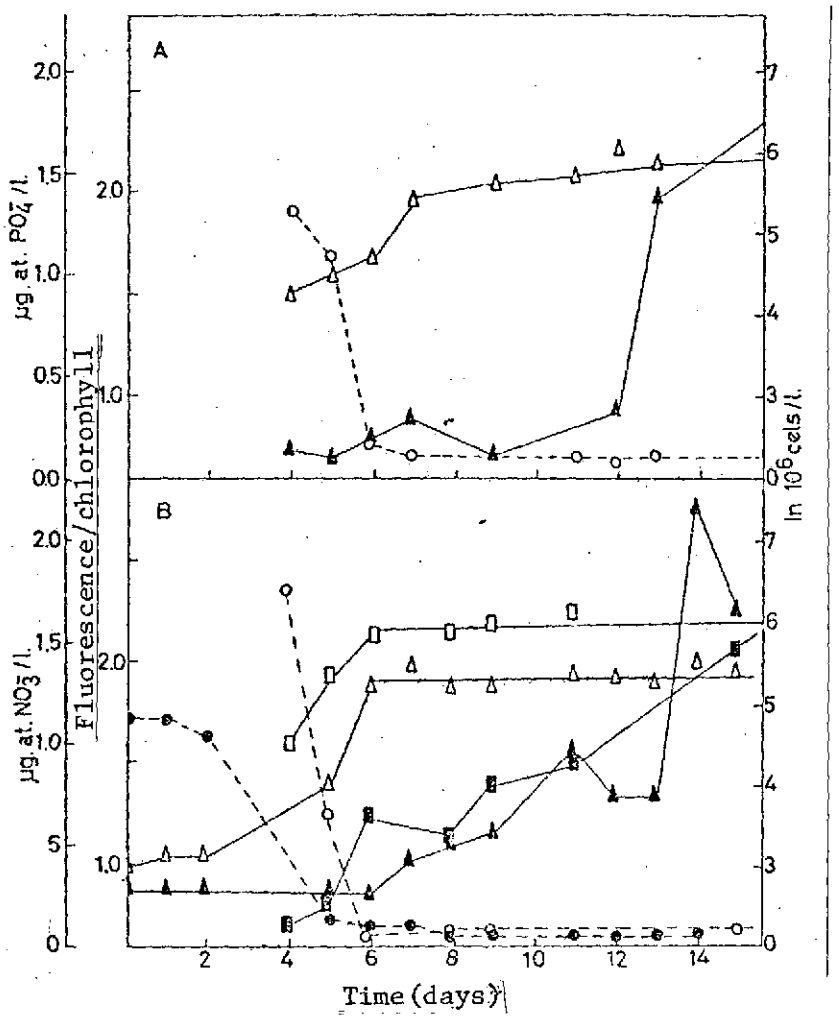

Figure 3. Change in the number of cells, △-△, □-□ ; phosphates and nitrates, ○-○, ●-● ; and fluorescence/chlorophyll ratio, ▲-▲, ■-■ in limited volume cultures. It was attempted to use this information on the results of the campaigns performed in northwestern Africa: Sahara I (Velasquez and Cruzado, 1973) and Atlor I (Estrada, 1973)

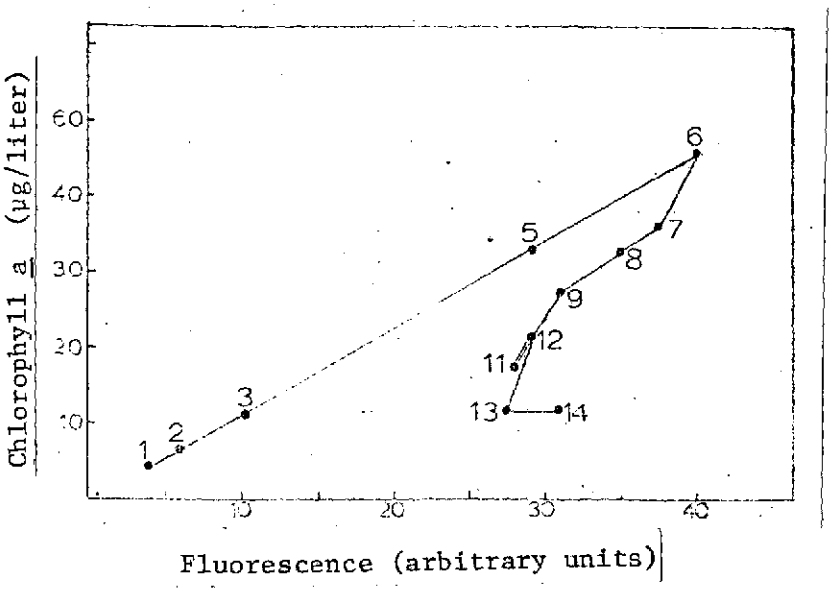

Figure 4. Change in the fluorescence/chlorophyll ratio in limited volume cultures. Nitrate as limiting component. Culture age in days.

Figure 3 shows the changes in the number of cells, in the concentration of the growth determining nutrient and in the fluorescence in vivo/chlorophyll a ratio during the experiments. It can be seen that the fluorescence/chlorophyll ratio increases with culture age. It can also be seen that in cultures limited in nitrate this ratio increases immediately after the cells have exhausted the nitrate in the medium; in contrast, in the cultures with /543
limiting phosphate this increase does not occur until after a period of time.

We conclude that nitrate affects this ratio more directly. Goedheer (1965) points out the difference in fluorescence intensity in cultures of different ages, as well as differences in the fluorescence spectrum. Brown (1967) notes that in Phaeodactylum tricornutum cultures, fluorescent emission increases with culture age. The term "culture age" in cells that reproduce by division, /544
and whose generation time is short, is somewhat ambiguous but it might be used to indicate different proportions of cells in different growth phases, be it for lack of nutrient or lack of light caused by increased culture density. Neither of these authors provides any additional information.

Figure 4 shows the changes in the fluorescence/chlorophyll a ratio /545
throught the culture's development. It can be seen that the ratio is constant during exponential culture growth, but that it changes -- favoring

fluorescence, as the culture reaches the stationary state. Fluorescence increases when the culture stops active growth, in perfect agreement with the hypothesis that the fluorescence of chlorophyll a competes with photosynthesis. For cultures with a less active growth, the photosynthetic activity will be less, and fluorescence per unit chlorophyll will be higher.

The fact that nitrate defficiency affects this ratio more drastically may indicate changes in the relative proportion of chlorophyll a components, whether fluorescent or not, or weakly fluorescent. In both cases exhaustion of nutrients affects the growth rate of the pigments per se.

INVESTIGATION OF THE FLUORESCENCE IN VIVO/CHLOROPHYLL a RATIO IN CELLS EXPOSED TO LIGHT OR DARKNESS

According to Brody and Brody (1958) the efficiency of energy transfer between the various photosynthesizing pigments varies, depending on the illumination to which the algae are exposed. Kautsky and Eberline (1939) observed, during measurements of the fluorescence intensity in chlorophyll in vivo that cells kept in darkness during a certain period of time, showed a rapid increase during the first few seconds of illumination; after this period the fluorescence decreased until a relatively stationary value was achieved. This fact -- known as photosynthesis induction curve -- has later been described by numerous authors (Lavorel, 1959; Lavorel, 1962; Butler, 1962; Rosenberg et al., 1964; Malkin and Kok, 1966; Papageorgiou and Govindjee, 1971, etc.)

The phenomenon of photosynthesis induction will occur also in cells that have been previously illuminated, provided they are placed in the dark for a short period of time. Malkin and Kok (1966) observed that the induction process is fully restored if the samples are left in the dark for 20 minutes and that in some cases, this restoration can be achieved in even less time. The illumination time needed to achieve maximum fluorescence is a function of temperature and exciting light intensity. Malkin and Kok (1966) found a time of 9 seconds at low light intensity and a temperature of 5°C. The ratio between the maximum and the stationary value increases with exciting light intensity. According to Rosenberg et al. (1964), the fluorescence in the stationary state is 53% of the maximum value observed. The peak in the curve is reached after 10 seconds and stationary state after 30 seconds. /546

The purpose of the experiments performed was to find out whether there were significant differences between fluorescence measurements taken during the day and at night, to take them into consideration when the conversion factor is calculated in oceanographic campaigns. This investigation was performed on laboratory results, for continuous cultures submitted to 8 hours of darkness and then 16 hours of illumination; also on results obtained on the ocean during the Mescal 1 campaign along the Baja California coast. /547

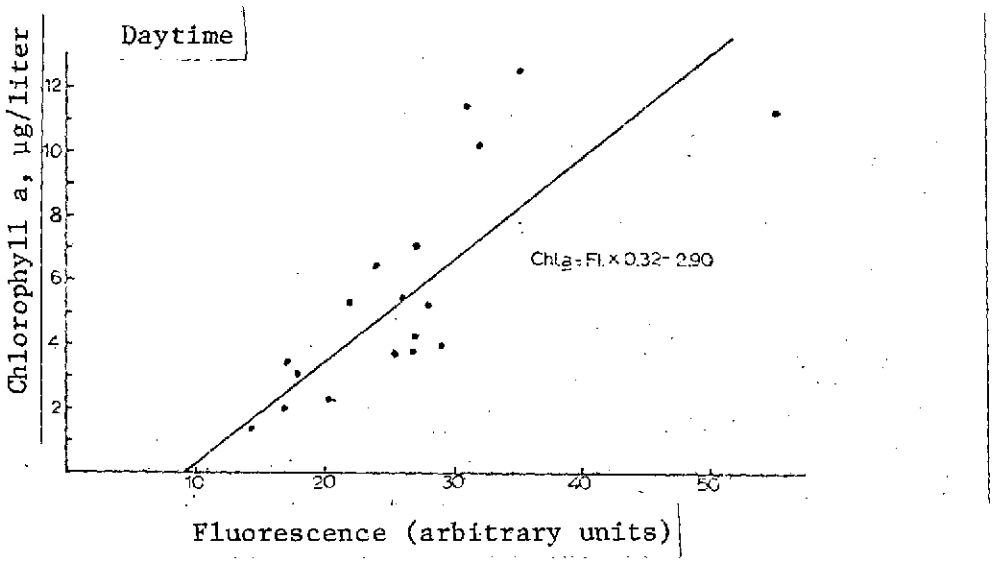

Figure 5. Chlorophyll/fluorescence ratio during the day, Mescal 1 Oceanographic Campaign

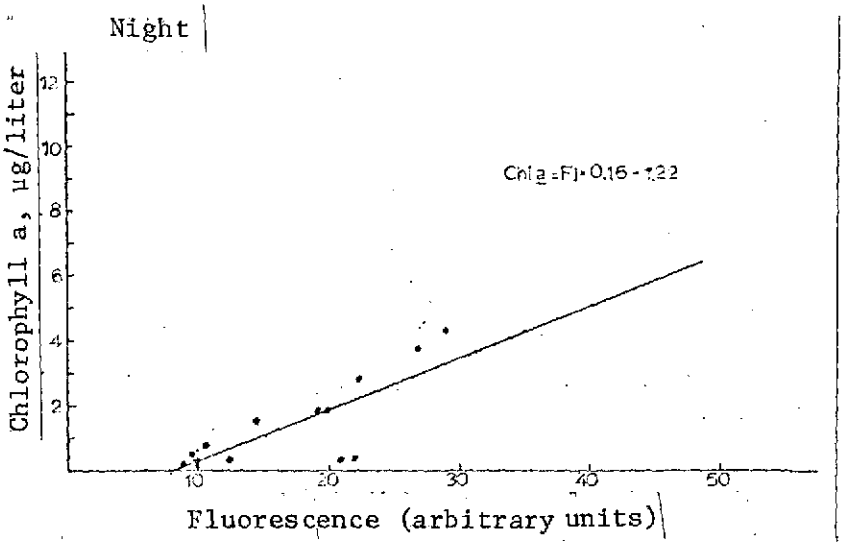

Figure 6. Chlorophyll/fluorescence ratio during the night, Mescal 1 Oceanographic Campaign

TABLE VII. AVERAGE AND (BETWEEN BRACKETS) STANDARD DEVIATION FOR THE RATIO FLUORESCENCE/CHLOROPHYLL a. CONFIDENCE LEVELS FOR THE HYPOTHESIS THAT THE DIFFERENCE BETWEEN THE AVERAGES IS DIFFERENT FROM ZERO

		Fl/Cl. a	*t*	Confidence level
Cycle 1	Day	1,262 (0,100)	2,354	95 %
	Night	1,367 (0,059)		
Cycle 2	Day	0,753 (0,149)	0,0718	50 %
	Night	0,806 (0,010)		
Cycle 3	Day	0,543 (0,056)	2,526	95 %
	Night	0,625 (0,002)		
Cycle 4	Day	0,456 (0,062)	0,9282	70 %
		0,480 (0,001)		

In the laboratory we measured the chlorophyll a concentration and the fluorescence during 24 continuous hours, in four experiments. The results (Table VII) clearly indicate that the fluorescence/chlorophyll a ratio is higher during the night. /548

The same observation was made from data collected during the campaign; water samples were taken at the fluorimeter exit, with the chlorophyll a concentration measured. The on-board fluorimeter is mounted in such a manner that the water pumped from the surface to the laboratory continuously flows through the fluorimeter, by means of a continuous flow cell. The tubing that carries the surface water to the laboratory is opaque, but prior to admission to the fluorimeter, the water passes through a degassing vessel exposed to the laboratory light, in order to prevent bubbles from entering the fluorimeter. It has been calculated that the time spent by the sample in this vessel is of the order of 5 seconds, while that spent in the fluorimeter itself is of tenths of seconds. It is hence likely that when the sample reaches the fluorimeter, the fluorescence is near the induction curve peak.

TABLE VIII. STATISTICAL PARAMETERS FOR THE REGRESSION LINES FLUORESCENCE/ CHLOROPHYLL a. Chlor. a = Fluorescence x B + A

	Correlation C	B	A	Standard error	N	t calcul.	t tabul. 99,9 %
Day samples	0,81	0,32	−2,90	0,090	20	3,55	2,55
Night samples	0,80	0,16	−1,22	0,055	14	2,90	2,68
Deep samples	0,78	0,56	0,90	0,141	27	3,97	2,48
Surface samples	0,85	0,38	−0,38	0,089	26	4,26	2,49

TABLE IX. CONFIDENCE LEVELS TO REFUTE THE HYPOTHESIS THAT THE SLOPES ARE IDENTICAL

	t calculated	Confidence level
Day Night	1.523	90 %
Surface Depth	1.084	85 %

During the same campaign, fluorescence in vivo was measured at the productivity stations, at the levels of 100%, 50%, 25%, 10% and 1% of light intensity penetration; chlorophyll a was determined spectrophotometrically in the same samples. The regression lines calculated for surface samples with 1% light (Table VII) clearly show that fluorescence due to chlorophyll a is higher in the depths than on the surface. This may well be due to either the effect of darkness or to differences in the pigment composition of these populations.

FLUORESCENCE IN VIVO/CHLOROPHYLL a RATIO IN DIFFERENT SPECIES

Strickland (1968) observed that the fluorescence per unit pigment depends strongly on the species. He investigated seven different species, finding variations of from 1 to 5 between species.

TABLE X. STATISTICAL PARAMETERS FOR THE REGRESSION LINES FLUORESCENCE/ CHLOROPHYLL a. (Chlor. a = Fluorescence x B + A)

/549

	C Correlation	B	A	Standard error	N	t calculated	Confidence level
Thalassiosira fluvialitis	0,73	3,29	18,50	1,36	12	2,41	95 %
Skeletonema costatum	0,66	1,25	66,80	0,672	8	1,87	90 %
Dunaliella tertiolecta	0,91	3,90	8,56	0,978	20	3,98	95 %

TABLE XI. CONFIDENCE LEVEL TO REFUTE THE HYPOTHESIS THAT THE SLOPES FOR EACH GROUP ARE IDENTICAL

Thalassiosira fluviatilis *Skeletonema costatum*	90 %
Thalassiosira fluviatilis *Dunaliella tertiolecta*	60 %
Dunaliella tertiolecta *Skeletonema costatum*	95 %

Three species were studied in this investigation, of which two were marine diatoms -- Skeletonema costatum and Thalassiosira fluviatilis -- and the third was Dunaliella tertiolecta, a marine chlorophycea. The regression lines for each species shown in Table X were calculated from the results obtained. The slopes for each species are significantly different. Table XI shows the confidence levels to confirm the hypothesis that they are not identical.

It can be clearly seen that proximity between slope values is not a function of philogenetic similarity in the species, since there is a larger difference between Thalassiosira fluviatilis and Skeletonema costatum, than there is with Dunaliella tertiolecta. This is comparable to Strickland's results (1968).

CONCLUSIONS

The validity of using fluorescence in vivo measurements as a measure of chlorophyll a can not be disputed, since a positive, significant correlation between both quantities was observed in all experiments.

Nevertheless, since the observed ratio is not constant and depends heavily on the cells' physiological characteristics, the precision of the estimate will be a function of the homogeneity of the area under investigation, and of whether all measurements were taken during the day or during the night. /550

We conclude that in order to obtain a correct estimation, it is necessary to calibrate the fluorimeter not only over a short period of time, but throughout the campaign and for both day and night, to obtain values that represent the conditions encountered.

The study performed during the MESCAL I campaign can be used as an example of the errors that can be incurred if these precautions are not taken. If the conversion factor calculated from samples taken during the day is applied to fluorescence measurements taken at night, then the error introduced in different fluorescence values can be significant (Figure 7). The same will happen if a factor derived from surface samples is used to calculate chlorophyll in depth samples.

We also conclude that due to the fact that fluorescence measurements depend very heavily on the conditions to which the sample was subjected prior to placing it in the fluorimeter and on the time elapsed to the reading, it is advisable to automate these measurements wherever possible, so that these conditions remain constant for all measurements. /551

Another important fact according to the results submitted with this investigation is that if the fluorescence measurements are performed with the appropriate precision, then the variations in the fluorescence/chlorophyll a ratio will reflect the specific phytoplankton composition, and the physiological characteristics of the population. Changes will be due to differences in pigment composition or differences in the efficiency of use and transfer of energy by the different pigments. The Turner fluorimeter (111-003) with the R-136 photomultiplier and a 110-921 filter measures the total fluorescence from 650 to 750 mμ. Within this wavelength range the

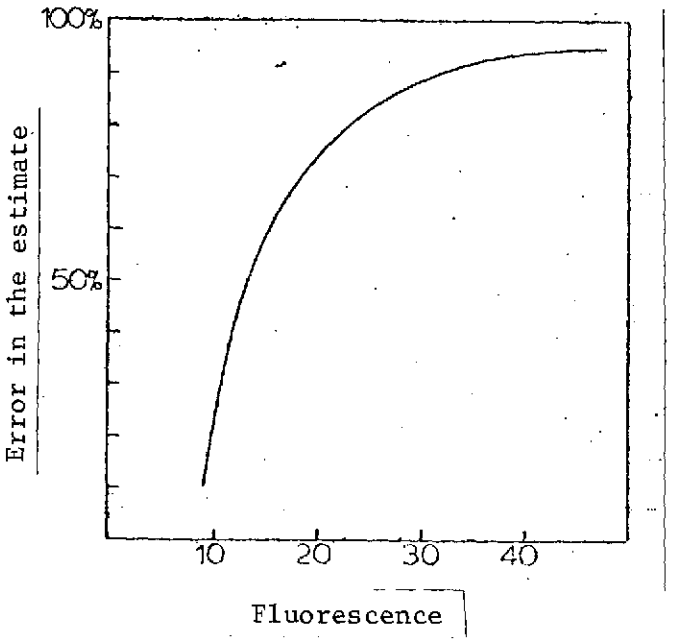

Figure 7. Error in the chlorophyll estimate when the calibration factor derived from daylight samples is applied to fluorescence measurements taken at night.

fluorescence may be due to the chlorophyll a fluorescence bands at 680-685 mμ and at 710 mμ (Rabinowitch and Govindjee, 1969; Brown, 1967), and if cyanophycea are present, to phycocyanin 656 (Goedheer, 1964). Since cyanophycea are algae found only in very limited ocean areas, in most oceanographic campaigns only the 685 mμ and 710 mμ fluorescence bands will be measured. As already mentioned, chlorophyll fluorescence competes with photosynthesis; hence an increases in fluorescence intensity per chlorophyll unit could indicate a decrease in photosynthesis activity, as the limited volume experiments clearly show.

Another interesting observation to be made from the experiments performed is that the fluorescence/chlorophyll a ratio is inversely proportional to the chlorophyll concentration per cell. This fact was observed in the experiments performed with Thalassiosira fluviatilis (Figure 8) and Skeletonema costatum (Figure 9). This fact has not been commented on in the literature as published to date, perhaps due to the fact that most authors who have investigated the fluorescence in vivo/chlorophyll relationship have

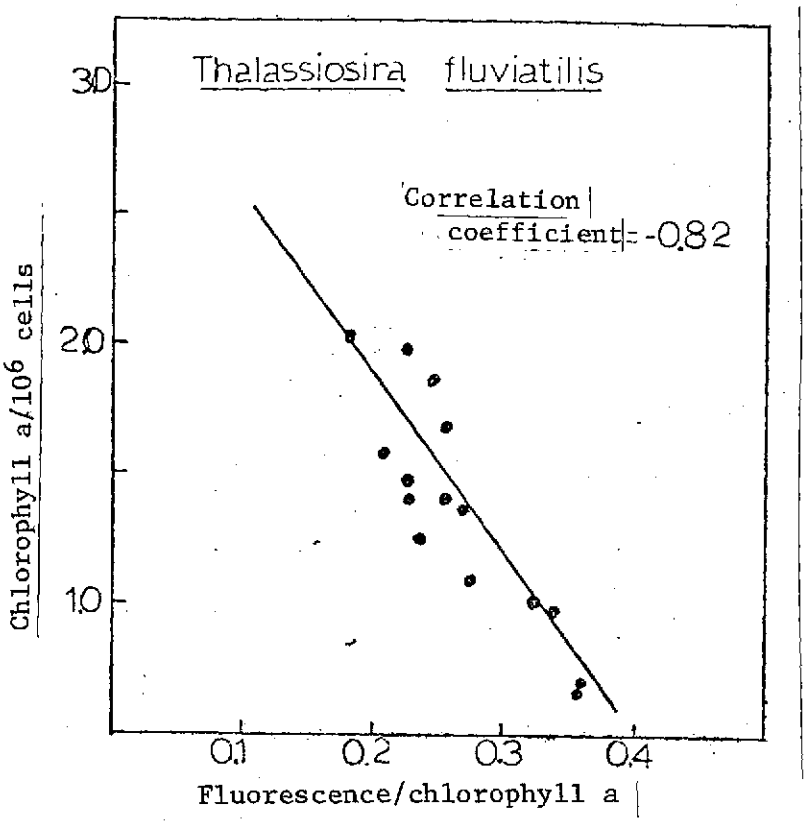

Figure 8. Relationship between chlorophyll concentration per cell and the fluorescence/chlorophyll a ratio.

not been primarily interested in cell characteristics per se, but in those of the pigments per se; furthermore, few investigations have been performed with diatoms. The cause of this phenomenon could be either that the quantity of pigments in photosystems I and II does not increase in the same proportion when the chlorophyll concentration increases (Brown, 1967), or that the transfer efficiency between the two systems increases when the chlorophyll molecules are more condensed (Brody, 1968). We do not have enough information to decide in favor of one or the other hypotheses.

Another fact to be considered is that chlorophyll pigments do not always parallel the cell growth rate (biomass), but rather exhibit their own,

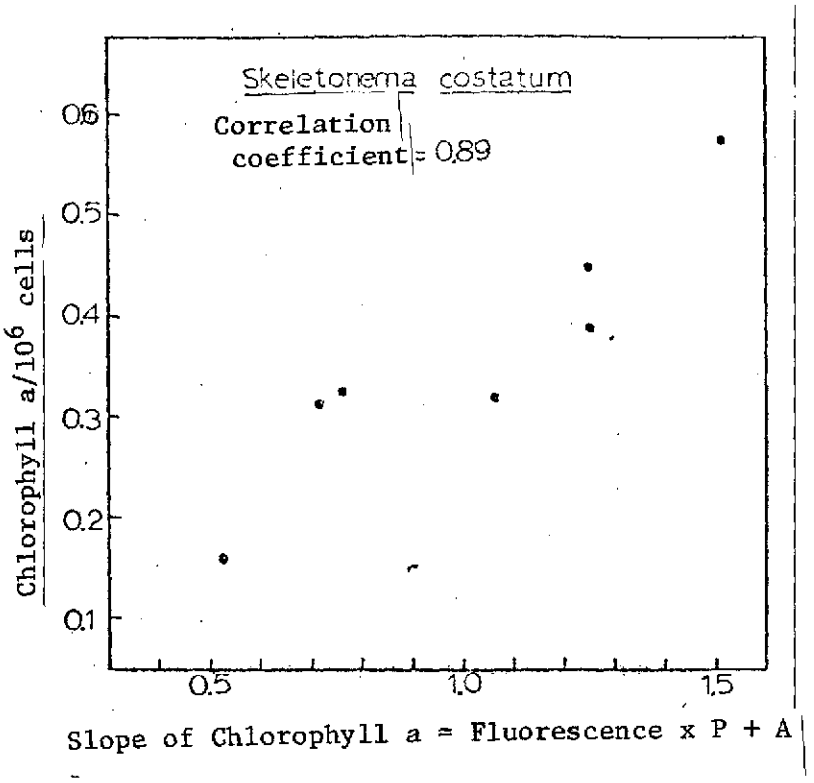

Figure 9. Relationship between chlorophyll concentration per cell and the fluorescence/chlorophyll a ratio.

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independent renewal rates, controlled by other factors (the cells' genetic characteristics, available nutrient concentration, etc.). Finally, the most significant conclusion is that a systematically performed study of the fluorescence/chlorophyll ratio during oceanographic research campaigns can provide significant information on the characteristics of the phytoplankton populations encountered.

One line of research that should be started would be the measurement also of the fluorescence spectra of the phytoplankton populations to be studied. With currently available automated techniques, such a study is not difficult to perform. It would only require the installation, on board ship, of a spectrofluorimeter; using the same sampling system used with particle counters, fluorescence emission and excitation spectra of the water under investigation could then be obtained continuously, every preset period of time (such as 5-10 minutes).

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